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COMBINED EFFECT OF ETHANOLIC LEAF EXTRACT OF *CARICA PAPAYA* AND *NEWBOULDIA LAEVS* ON THE CEREBELLUM OF ALLOXAN-INDUCED DIABETIC MALE WISTAR RAT

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ABSTRACT

Objective: This study was carried out to investigate the combined effect of ethanolic leaf extracts of *C. papaya* and *N. laevis* on the cerebellum of alloxan-induced diabetic male wistar rats.

Methodology: Forty (40) male wistar rats weighing 150-180g were procured and acclimatized for two weeks, after which they were divided into eight (8) groups of five (5) rats each, and were housed in cages. The groups were designated as groups A - H. Group A served as the control group, and received distilled water only. Animals in groups B – H were induced with diabetes using alloxan. The diabetic group B did not receive any treatment throughout the experiment, while the diabetic groups C - H received 400mg/kg of *C. papaya* leaf extract, 600mg/kg of *C. papaya* leaf extract, 400mg/kg of *N. laevis* leaf extract, 600mg/kg of *N. laevis* leaf extract, 200mg/kg of *C. papaya* + 200mg/kg of *N. laevis*, and 300mg/kg of *C. papaya* + 300mg/kg of *N. laevis* leaf extract respectively for 21 days through oral route with the aid of oral gastric tube. On the 22nd day, the animals were sacrificed via chloroform inhalation, and cerebellums were harvested for histological studies.

Result: The histopathological findings showed molecular layer (ML), granular layer (GL) and well outlined pyramidal cell within the purkinje layer (PL) in group A; severe degeneration with severe fatty change (FC) severe vacoulation (V) of purkinje cells layer pyknotic (P) pyramidal cell and aggregate of inflammatory cell (AIC) within the hemorrhagic (H) area in group B; normal histological feature with well outlined pyramidal cells (PC) in group C; moderate regeneration with moderate focal areas of hemorrhage (H) and moderate pyknotic (P) pyramidal cell in group D; moderate regeneration with mild vacoulation (V) and mild pyknotic (P) pyramidal cell in group E; moderate regeneration moderate increase in the number of pyramidal cells (PC) in group F; mild regeneration with moderate vacoulation (V), pyknotic (P) prymadial cell, mild cytoplasmic ground glass appearance within the molecular layer (ML) and focal area of hemorrhage (FAH) in group G; and mild regeneration with moderate vacoulation (V) and moderate fatty changes within the molecular layer (ML) in group H of the cerebellums of the alloxan-induced wistar rats.

Conclusion: Combined leaf extracts of *Carica papaya* and *Newbouldia laevis* have anti-diabetic and ameliorating effect on the histology of cerebellum of alloxan-induced male wistar rats.

Keywords: *Carica papaya, Newbouldia laevis,* Cerebellum

1.0 INTRODUCTION

About 422 million people worldwide have diabetes, the majority living in low-and middle-income countries, and 1.5 million deaths are directly attributed to diabetes each year [1]. Both the number of cases and the prevalence of diabetes have been steadily increasing over the past few decades [1]. Diabetes, which is a disorder in which the body does not produce enough or respond normally to insulin, causing blood sugar (glucose) levels to be abnormally high [2], affects all cerebellar layers, as well as the myelin sheath and vascular structures in the cerebellum [3]. Type 2 diabetes mellitus (T2DM) is a risk factor for Alzheimer's disease (AD) and vascular dementia [4, 5 and 6]. It causes emotional abnormalities and multiple cognitive dysfunctions, such as executive function and visual space [7]. Brain network disorders and abnormal neuronal activity are the neural bases of cognitive impairment. Studies have shown that disruption of the default-mode network (DMN) may be related to episodic memory impairment [8] and depression [9], whereas disruption of the executive control network (ECN) may lead to reduced working memory [10] in patients with T2DM. In addition, T2DM studies have demonstrated abnormal neuronal activity in the core regions (posterior parietal and occipital cortex) of the visuospatial network (VSN) [11, 12, and 13]. Long-term effects of diabetes on the brain are manifested at structural, neurophysiological, and neuropsychological level, and multiple pathogenic factors appear to be involved in the pathogenesis of the cerebral dysfunctioning in diabetes like the hypoglycemic episodes, cerebrovascular alterations, the role of insulin in the brain, and the mechanisms of hyperglycemia induced damage [14]. Moreover, the emerging view is that the diabetic brain features many symptoms that are best described as accelerated brain ageing [15]. A common theory, for aging and for the pathogenesis of cerebral dysfunctioning in diabetes, relates cell death due to oxidative stress mediated by free radicals [16]. Thus, hyperglycemia reduces antioxidant levels and concomitantly increases the production of free radicals. These effects contribute to tissue damage in diabetes mellitus, leading to alterations in the redox potential of the cell with subsequent activation of redox-sensitive genes [17]. The brain is especially vulnerable to oxidative damage as a result of its high oxygen consumption rate, abundant lipid content, and relative paucity of antioxidant enzymes as compared to other tissues. Neuronal cells are particularly sensitive to oxidative insults, and therefore reactive oxygen species (ROS) are involved in many neurodegenerative processes such as diabetes [18, 10, and 20]. Although under normal physiological conditions a balance exists between the production of ROS and the antioxidant mechanisms, it has been shown that in aging tissues oxidative stress increases due to, among others, decreased activity of antioxidant enzymes [21]. There is a globally agreed target to halt the rise in diabetes and obesity by 2025 [1].

Plants which possess therapeutic properties, or exert beneficial pharmacological effect on the human body and animal body are called medicinal plant [22]. Such plants include *Carica papaya* and *Newbuldia laevis*. *C. papaya* is one of the accepted twenty two species in the genus *Carica* of the family *Caricaceae* that originate in the tropics of the Americas, perhaps from Central America and southern Mexico [23]. It is a small, sparsely branched tree, usually with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk [24]. Its lower trunk is

conspicuously scarred where leaves and fruit are borne, and the leaves are large, 50–70 cm (20–28 in) in diameter, deeply palmately lobed, with seven lobes [24]. Their leaves have been used as a treatment for malaria [25], an abortifacient, a purgative, or smoked to relieve asthma in traditional medicine [23]. Also, the leaves reduce symptoms of asthma, worming and dysentery [26, 27], remedy cancer and infectious diseases [27], accelerates wound healing [28, 29], exhibit vasodilating and exhibit antioxidant effects, both being associated with cardiovascular risk reduction [26] and are useful in the treatment of diabetes in Nigeria [30]. According to Gray *et al*, [31] the leaf extract reduced glucose levels in alloxan induced diabetes suggesting that *Carica papaya* leaves might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism. Besides their hypoglycemic properties [29], different parts of *C. papay*a are used in Mexican folk medicine to treat various diseases such as diarrhea, inflammation and diabetes [29, 32]. *C. papaya* has also been attributed to the following properties - antioxidant activity, immunomodulatory, hypoglycemia and hypolipidemic [33] and hepatoprotective [34, 35]. *C. papaya* leaf extract may be beneficial to diabetic patients, helpful in the prevention of diabetic complications by dyslipidemia improvement [36], used for nerve pains (neuralgia) and elephantoid growths [37].

N. laevis belongs to the family *Bignoniaceae* in the order *Bignoniae.* It is a genus of one species with a medium size angiosperm tree that grows up to 7-8 m. It can also be a shrub of about 3 m and widely distributed in the tropics. It is shrubby or erect with vertically ascending branches. It is used in folkloric medicine to treat a number of diseases. Some of which include the following: the leaves and roots are boiled and used to treat earaches, sore foot, chest pain, fever, convulsion and epilepsy in children [38, 39], diarrhea [40]. The roots are used to treat arthritis, malaria and general malady and worms [41]. The leaves are used as decoction for eye wash in conjunctivitis and also as chieftaincy leaf in Yoruba land [41]. The stem bark is used for toothache, febrifuge, stomach and skin infections [38, 41]. Recently, the flowers and leaves have been used in the treatment of diabetes [39, 40, 42, and 43] respectively. It is also used to stop vaginal bleeding in threatened abortion [41] and had shown strong antioxidant activity [44]. According to Osigwe *et al*, [45] *N. laevis* leaf possesses the ability of managing hyperglycemia, improve haematological and biochemical derrangements in alloxan induced-diabetic rats, control muscle wasting and induce adipogenesis [45]. *N. laevis* leaf and stem have anti-diabetic properties [46]. Anaduaka *et al*, [49] reported that the ethanol extracts of the leaves and stem of *N. laevis* possess hepatoprotective properties for curbing oxidative stress complication. Kolawole *et al*, [42] in their research reported that the ethanolic extract of the leaves of *N. laevis* possesses anti-diabetic properties and that it can prevent the complications of diabetes that result from glycation of hemoglobin and lipid peroxidation. The leaf extract of the *N. laevis* has also been reported to lower blood glucose level in diabetic rats [40].

Therefore this study was carried out to investigate the effect of combined leaf extracts of *C. papaya* and *N. laevis* on the cerebellum of alloxan-induced diabetic male wistar rats since no work has been carried out on this.

2.0 MATERIALS AND METHODS

2.1 Animal procurement, care and treatment

Forty (40) male wistar rats weighing between 130g to 180g were procured and housed at the Animal house of Anatomy Department, Abia State University; Uturu with wire gauze cages in a well-ventilated area, were maintained under standard laboratory conditions of temperature ($22+2^0$C), relative humidity (55-65%) and 12 hours light/dark cycle. They were

fed with standard commercial pellet diet and water *ad libitum* and were also acclimatized for two weeks before the experiment. Their health statuses were closely monitored before and during the experiment. All procedures were carried out in strict accordance with the Institutional guidelines on the care and use of experimental animals.

2.2 Collection, identification and preparation of plant material

Fresh leaves of *C. papaya* and *N. lavis* leaves were plucked from Nkporo in Ohafia L.G.A., Abia State, and were authenticated at Herbarium unit, Botany Department, Abia State University, Uturu, Abia State. The leaves were air dried and crushed using laboratory blender. Extractions were done using ethanol. The crude ethanol extracts were kept in an air-tight container and stored in a refrigerator at 4^0C until time of use. At the time of use, the ethanol extracts were filtered into a stainless basin with a white cloth and placed in a water bath so as to dry up the ethanol. 250mg of these extracts /kg body weight were dissolved in 10mls of distilled water and were administered to the animals.

2.3 Induction of diabetes

The rats were divided into non-diabetic control group and experimental groups. The baseline blood glucose level of the experimental group to be inducted was determined before the induction of diabetes. The rats were allowed to fast over night prior to injection of alloxan and diabetes was induced by intra-peritoneal administration of 150mg of alloxan per kg body weight of rat (150mg/kg body weight) [48]. After the induction, the rats were allowed to have free access to the same feed and water. After 72 hours, blood samples obtained through tail tip puncture of the rats were used to confirm diabetes in the rats by testing for hyperglycemia using Glucometer. Diabetes was confirmed at fasting blood glucose levels greater than 200mg/dl [49].

2.4 Experimental protocol

The animals were grouped into eight (8) groups of five (5) rats each. Different doses of the leaf extracts were administered via oral route with the aid of oral gastric tube as shown below:

Group A	The control group + distilled water.
Group B	Diabetic group + No treatment
Group C	Diabetic + 400mg/kg of *C. papay* leaf extract.
Group D	Diabetic + 600mg/kg of *C. papay* leaf extract.
Group E	Diabetic + 400mg/kg of *N. laevis* leaf extract.
Group F	Diabetic + 600mg/kg of *N. laevis* leaf extract.
Group G	Diabetic + 200mg/kg of *C. papaya* and 200mg/kg of *N. laevis* leaf extracts.
Group H	Diabetic + 300mg/kg of *C. papaya* and 300mg/kg of *N. laevis* leaf extracts.

2.5 Sample collection and analysis

The extracts were administered for twenty one (21) days. On the 22nd day, the animals were sacrificed by anaestethizing under chloroform vapour and dissected. Cerebella organs were harvested from the wistar rats, and were fixed in 10% formal saline for four hours. This was followed by histological and histochemical methods of tissue processing.

3.0 RESULTS
Histopathological findings

Micrograph 1 is the result of microscopic examination of the animals in Group A1 & 2 (Control) sections of cerebellum (X100 X400/(H/E) showing normal cerebellum with molecular layer (ML), granular layer (GL) and well outlined pyramidal cell within the purkinje layer (PL).

Micrograph 2 is the result of microscopic examination of the animals in Group B1 & 2 sections of cerebellum induced with alloxan only without treatment (X100 X400) (H/E) showing severe degeneration with severe fatty change (FC) severe vacoulation (V) of purkinje cells layer pyknotic (P) pyramidal cell and aggregate of inflammatory cell (AIC) within the hemorrhagic (H) area.

Micrograph 3 is the result of microscopic examination of the animals in Group C1 & 2 sections of cerebellum induced with diabetes and treated with 400mgkg of *C. papaya* (X100 X400) (H/E) showing normal histological feature with well outlined pyramidal cells (PC).

Micrograph 4 is the result of microscopic examination of the animals in Group D1 & 2 sections of cerebellum induced with diabetes and treated with 600mg\kg of *C. papaya* leaf extract (X100 X400) (H/E) showing moderate regeneration with moderate focal areas of hemorrhage (H) and moderate pyknotic (P) pyramidal cell.

Micrograph 5 is the result of microscopic examination of the animals in Group E1 & 2 sections of cerebellum induced with diabetes and treated with 400mg\kg of *N. laevis* leaf extract (X100 X400) (H/E) showing moderate regeneration with mild vacoulation (V) and mild pyknotic (P) pyramidal cell in GE2.

Micrograph 6 is the result of microscopic examination of the animals in Group F1 & 2 sections of cerebellum induced with diabetes and treated with 600mg\kg of *N. laevis* (X100 X400) (H/E) showing moderate regeneration moderate increase in the number of pyramidal cells (PC**).**

Micrograph 7 is the result of microscopic examination of the animals in Group G1 & 2 sections of cerebellum induced with diabetes and treated with 200mg\kg *C. papaya* + 200mg\kg of *N. laevis* (X100 X400) (H/E) showing mild regeneration with moderate vacoulation (V), pyknotic (P) prymadial cell, mild cytoplasmic ground glass appearance within the molecular layer (ML) and focal area of hemorrhage (FAH).

Micrograph 8 is the result of microscopic examination of the animals in Group H1 & 2 sections of cerebellum induced with diabetes and treated with 300mg\kg of *C. papaya* + 300mg\kg of *N. laevis* (X100 X400) (H/E) showing mild regeneration with moderate vacoulation (V) and moderate fatty changes within the molecular layer (ML).

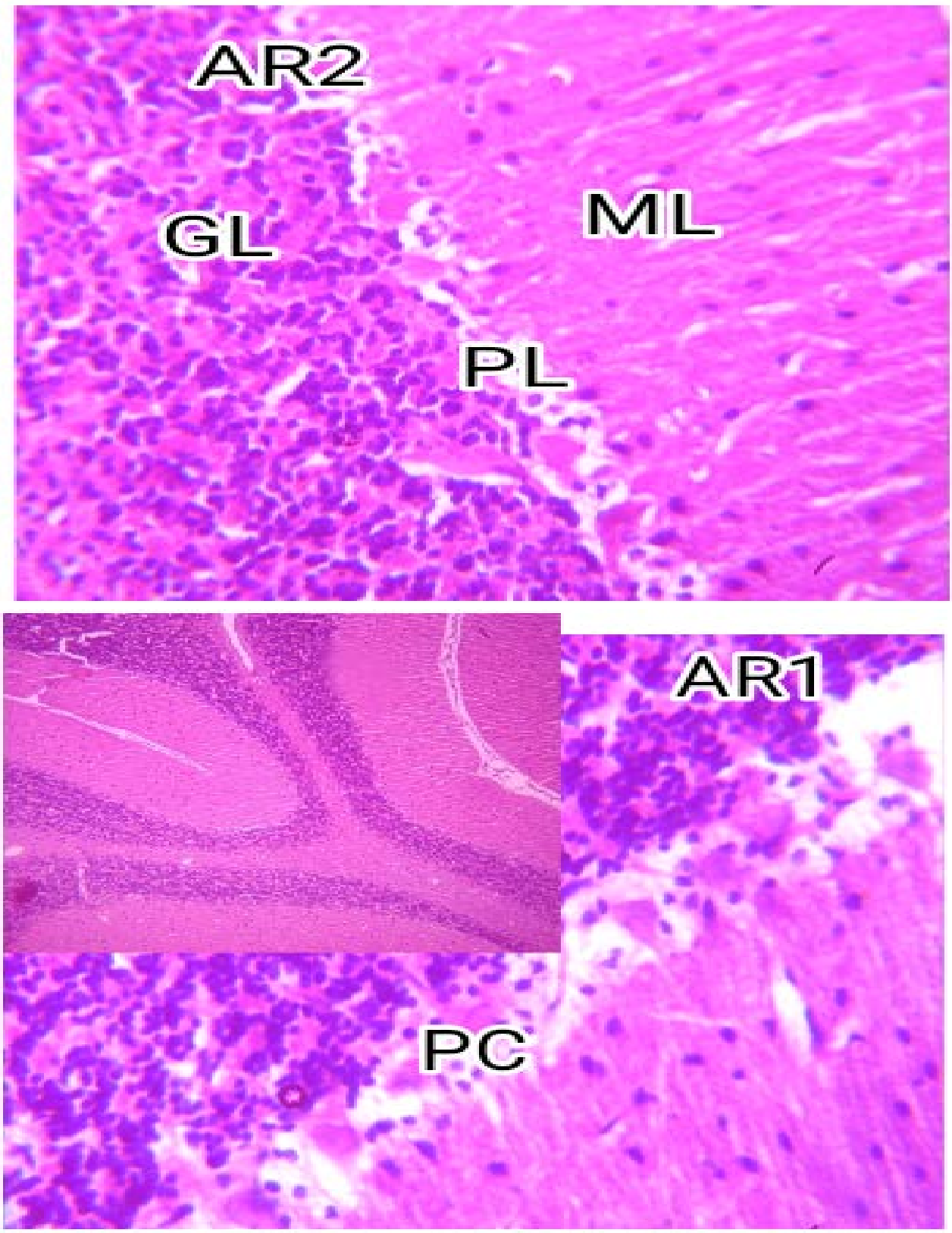

Figure 1: Micrograph 1 showing normal cerebellum with molecular layer (ML), granular layer (GL) and well outlined pyramidal cell within the purkinje layer (PL).

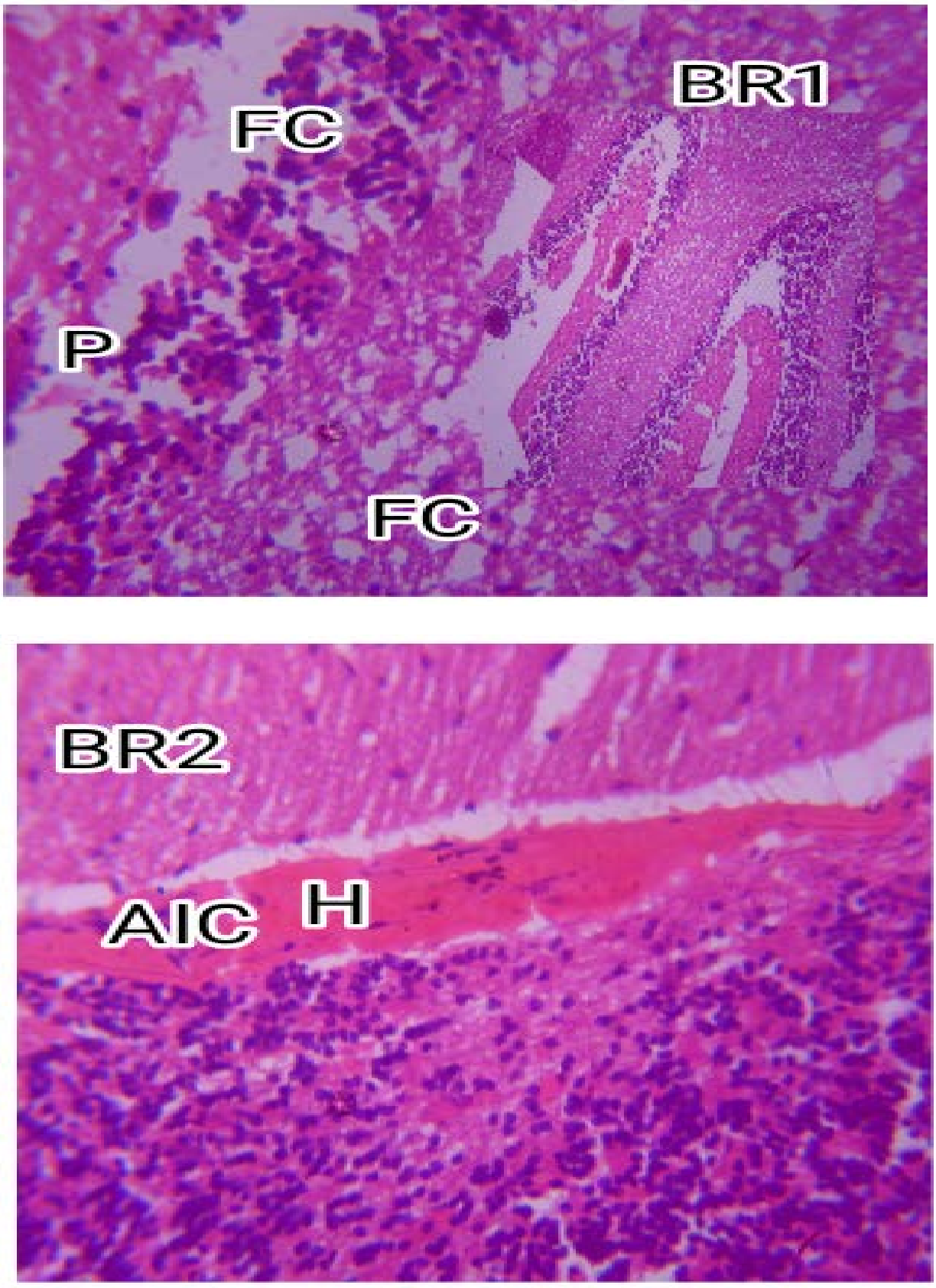

Figure 2: Micrograph 2 showing sever degeneration with severe fatty change (FC) severe vacoulation (V) of purkinje cells layer pyknotic (P) pyramidal cell and aggregate of inflammatory cell (AIC) within the hemorrhagic (H) area.

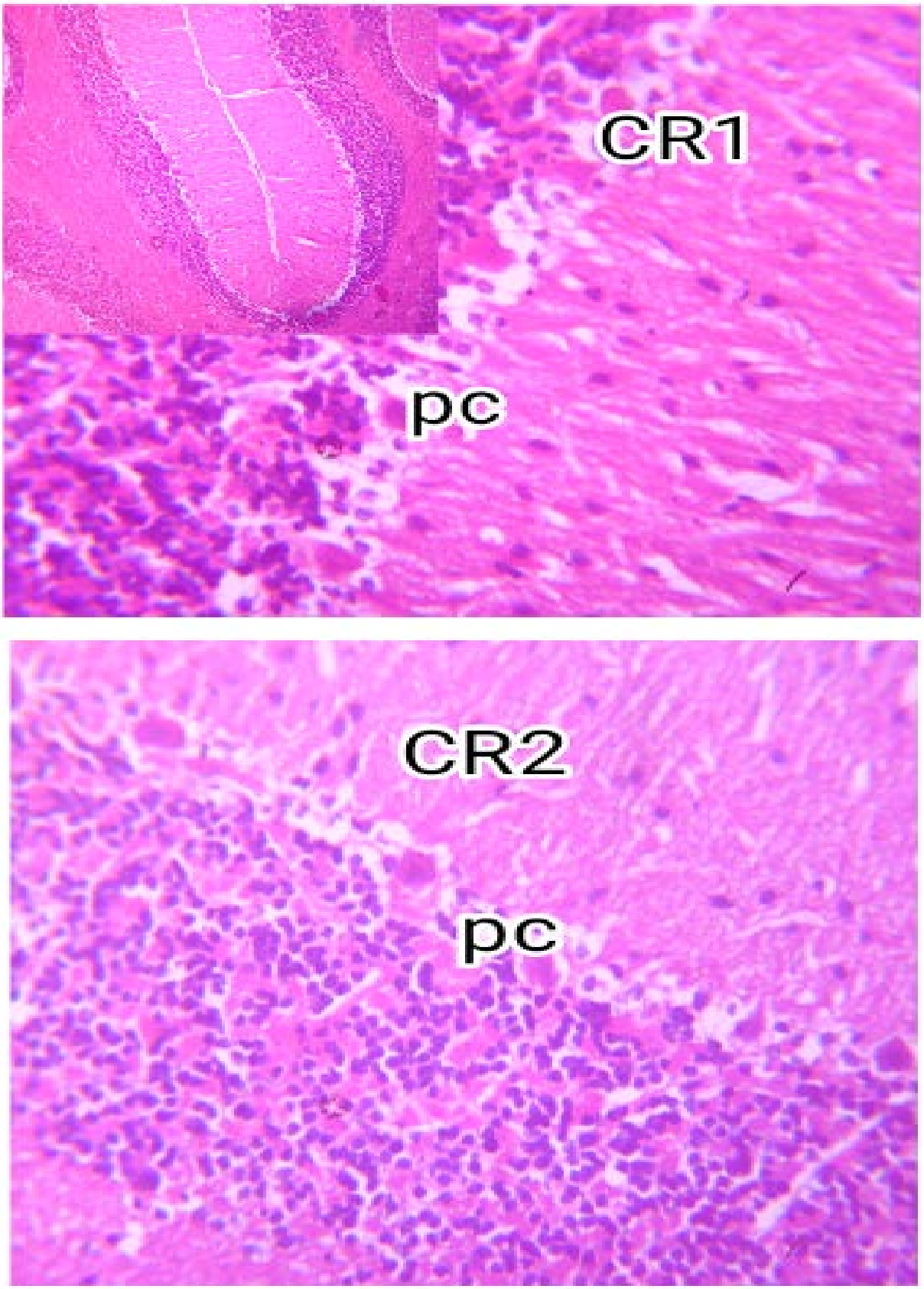

Figure 3: Micrograph 3 showing showing normal histological feature with well outlined pyramidal cells (PC).

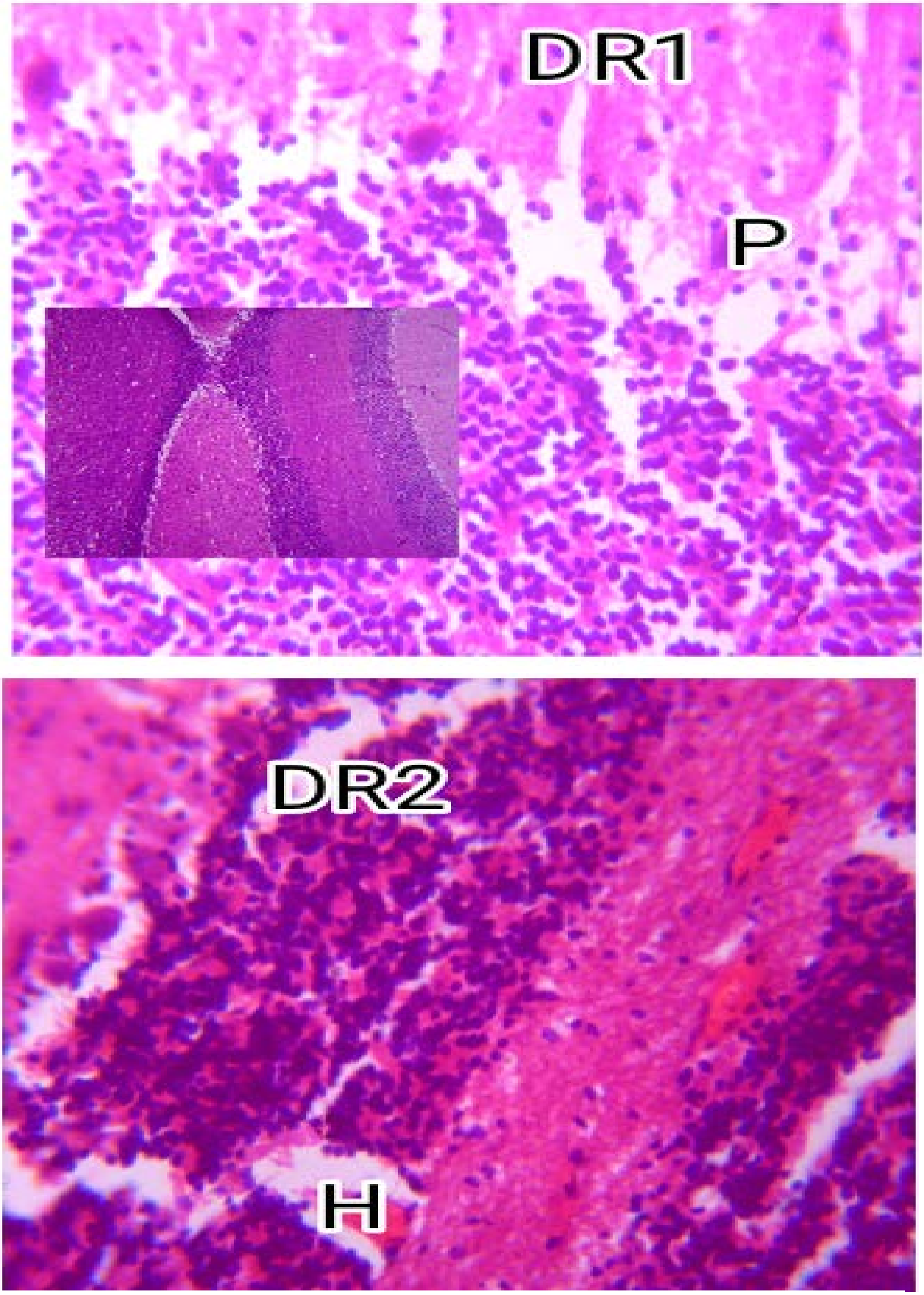

Figure 4: Micrograph 4 showing moderate regeneration with moderate focal areas of hemorrhage (H) and moderate pyknotic (P) pyramidal cell.

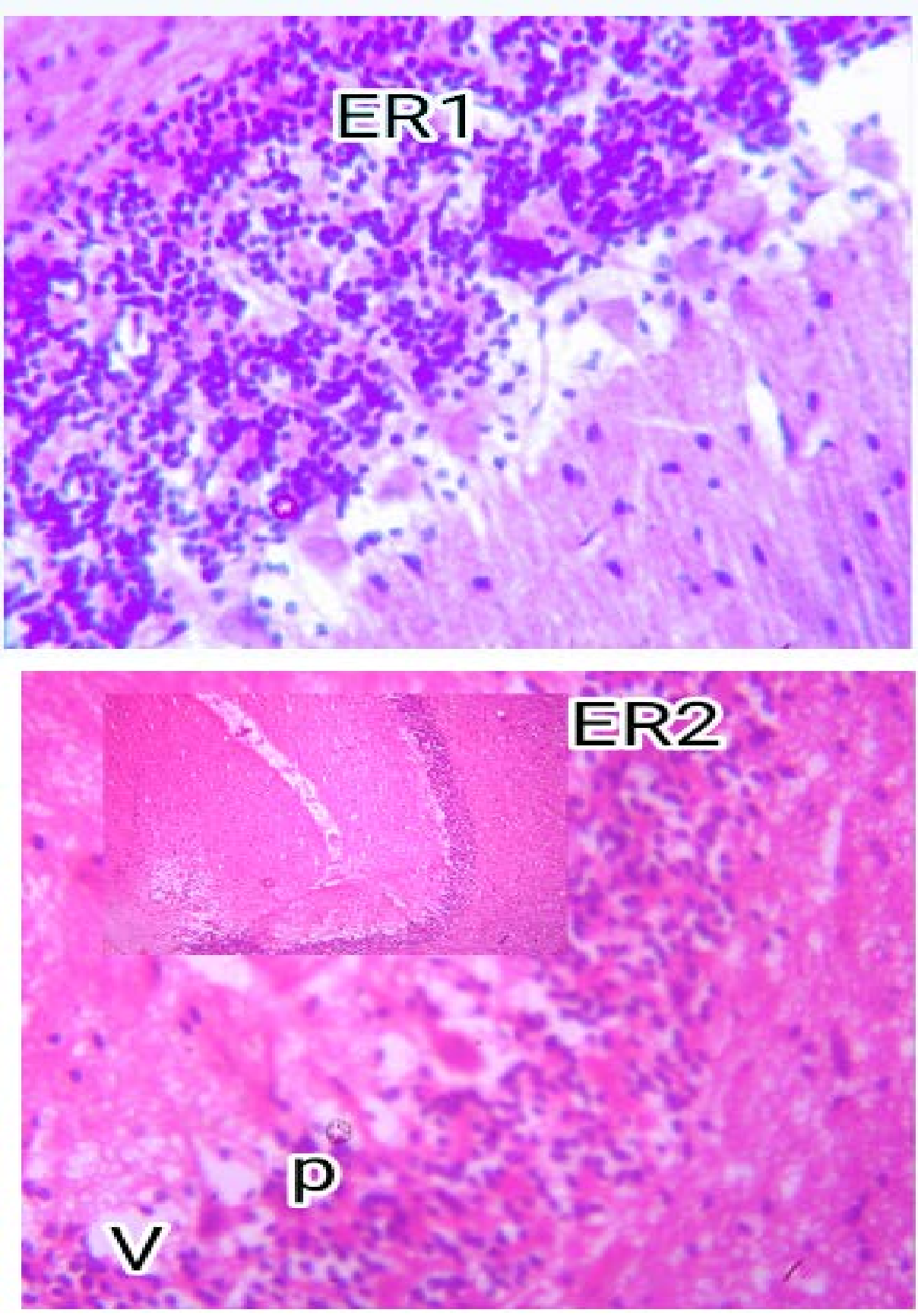

Figure 5: Micrograph 5 showing moderate regeneration with mild vacoulation (V) and mild pyknotic (P) pyramidal cell in GE2.

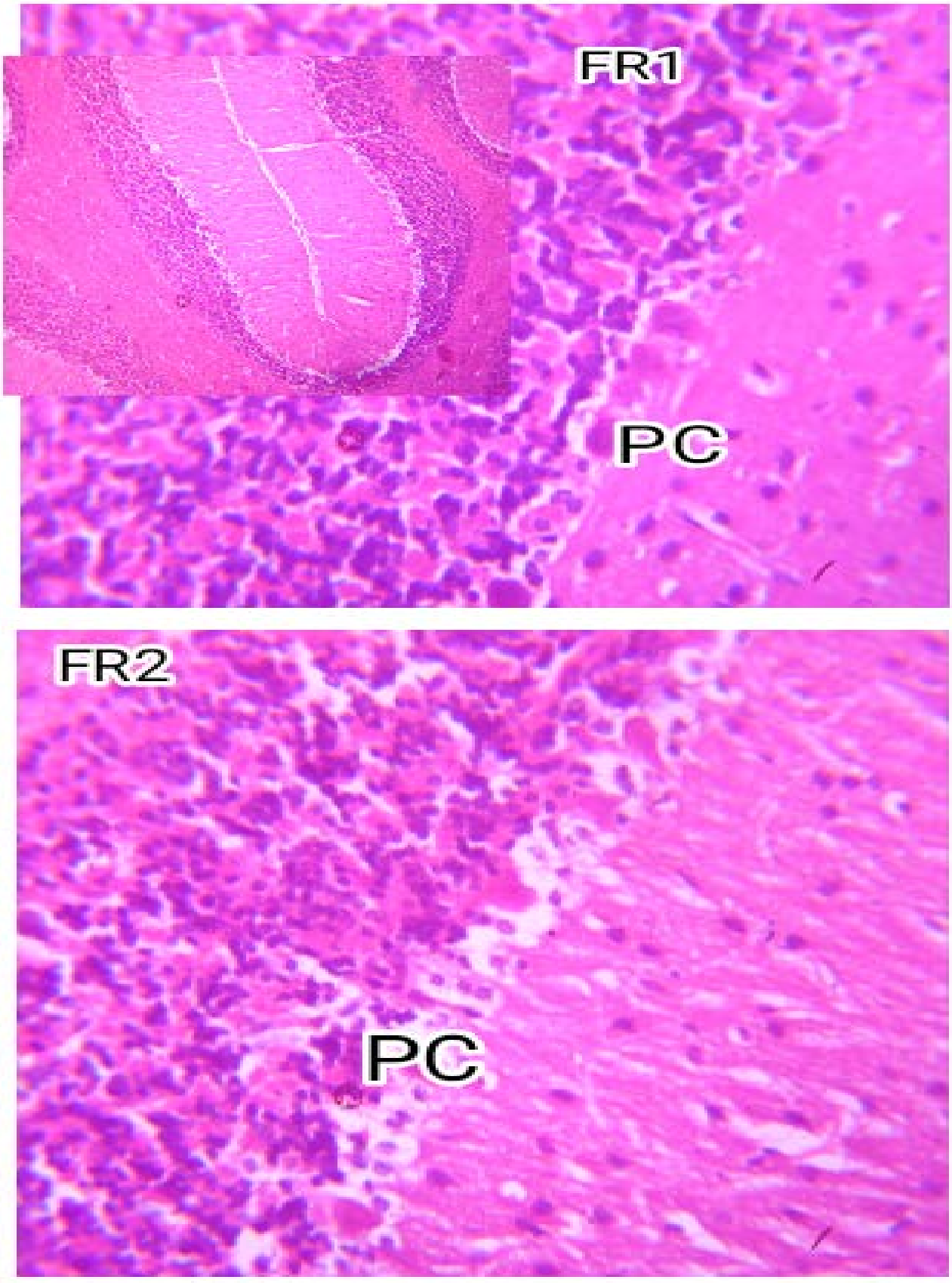

Figure 6: Micrograph 6 showing moderate regeneration moderate increase in the number of pyramidal cells (PC**).**

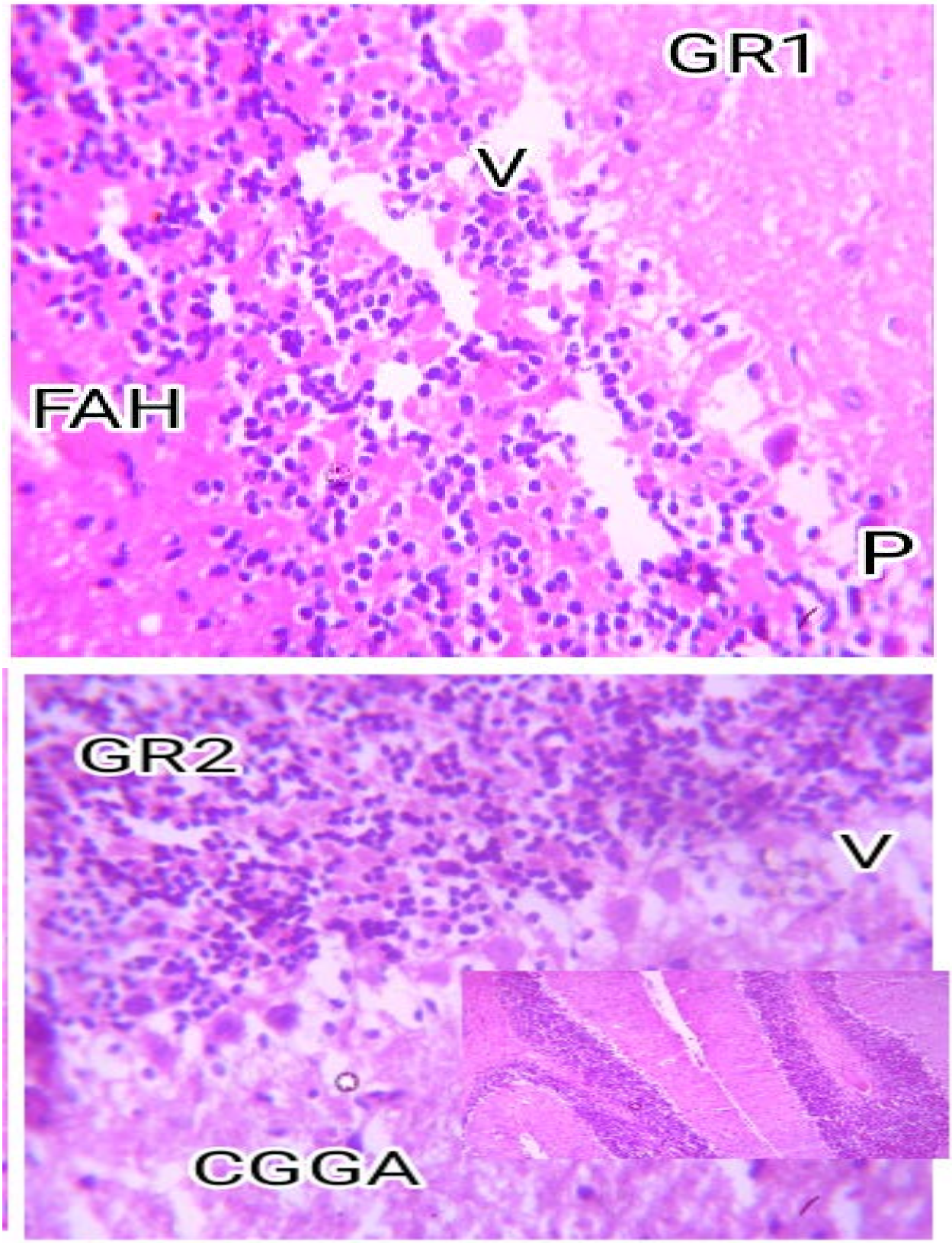

Figure 7: Micrograph 7 showing mild regeneration with moderate vacoulation (V), pyknotic (P) prymadial cell, mild cytoplasmic ground glass appearance within the molecular layer (ML) and focal area of hemorrhage (FAH).

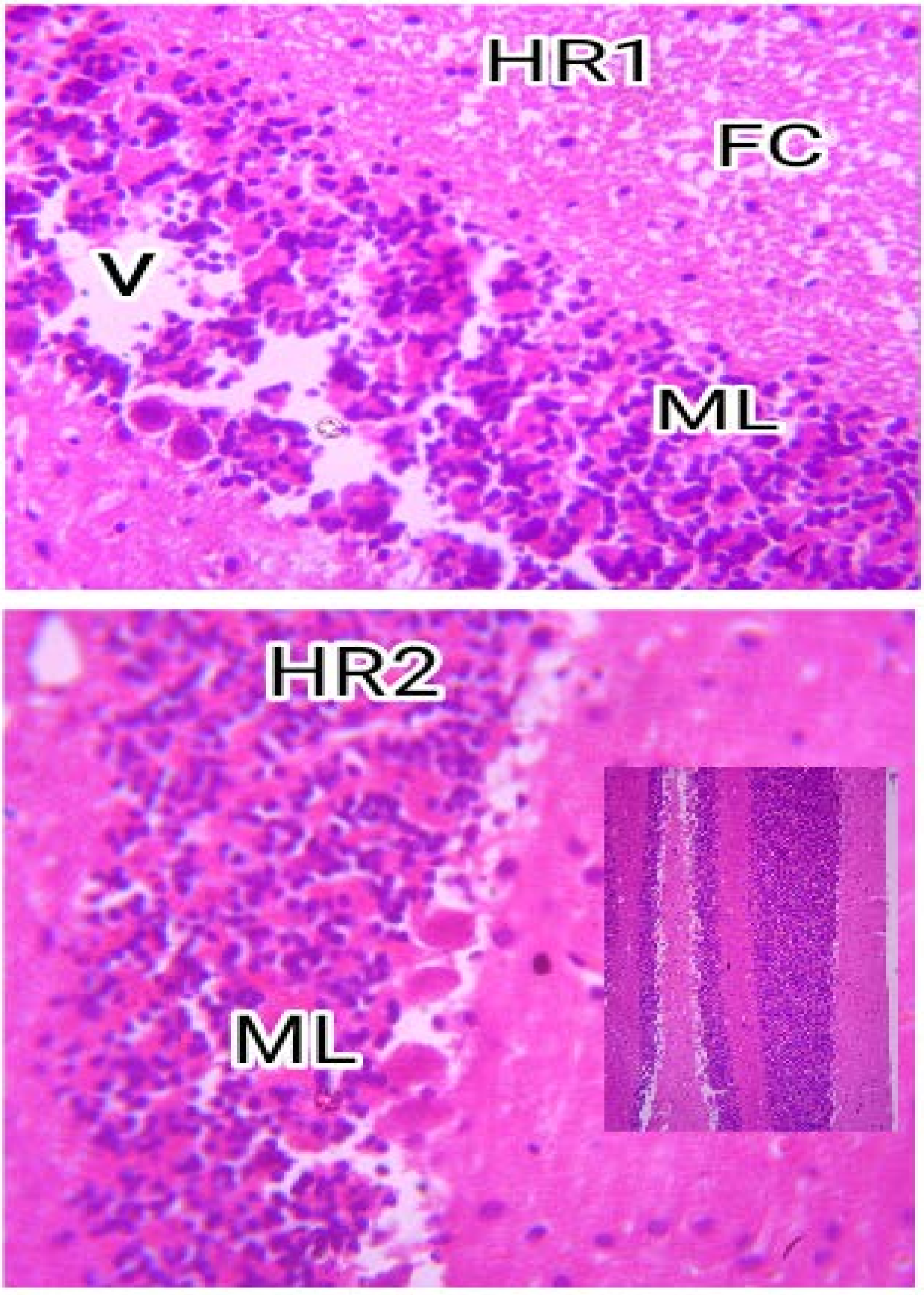

Figure 8: Micrograph 8 showing mild regeneration with moderate vacoulation (V) and moderate fatty changes within the molecular layer (ML).

4. DISCUSSION

Diabetes alters cerebral metabolism, structure, and function [50]. Most of the morbidity and mortality associated with diabetes is due to the development of the complications of the disease. Macrovascular complications such as coronary artery disease and stroke are a common cause of death in people with diabetes [50].

Micrograph 1 showed normal cerebellum with molecular layer (ML), granular layer (GL) and well outlined pyramidal cell within the purkinje layer (PL) which is in line with the histology of a normal cerebellum. According to Snell [51], the cerebellar cortex forms a series of deeply convoluted folds or folia supported by branching central medulla of white matter. Its cortex consists of three distinct layers, namely, the molecular layer, ganglionic or Purkinje cell layer and granular layer. The molecular layer consists of two main types of neurons - stellate cells and basket cells, which are scattered among dendritic ramifications and numerous thin axons that run parallel to the long axis of the folia; while the Purkinje cell layer is formed of a single row of large Purkinje cells with their axons of providing the only efferent pathway to the deep cerebellar nuclei, and thus constitute the sole output of all motor coordination in the cerebellar cortex [51]. The granular layer is densely populated by small granule cells with dark-staining nuclei and scanty cytoplasm [51].

The result of sever degeneration with severe fatty change (FC) severe vacoulation (V) of purkinje cells layer pyknotic (P) pyramidal cell and aggregate of inflammatory cell (AIC) within the hemorrhagic (H) area seen in micrograph 2 could be due to diabetes caused by the induced-alloxan. According to Lenzen *et.al*, [52] alloxan monohydrate induces diabetes in rats by destroying the insulin producing beta-cells of the pancreas causing cell necrosis. However, the results of these researchers suggest that the cerebellar morphological alterations that were observed during the early stages of treatment with alloxan may be more related to the toxic action of these drugs than to the effects of diabetes mellitus [53]. It has also been revealed that diabetes alters cerebral metabolism, structure, and function [50], and long-term effects of diabetes on the brain are manifested at structural, neurophysiological, and neuropsychological level; and multiple pathogenic factors appear to be involved in the pathogenesis of the cerebral dysfunctioning in diabetes like the hypoglycemic episodes, cerebrovascular alterations, the role of insulin in the brain, and the mechanisms of hyperglycemia induced damage [14].

Micrographs 3 and 4 treated with 400mg/kg and 600mg/kg of *C. papaya* leaf extracts showing normal histological feature with well outlined pyramidal cells (PC) and moderate regeneration with moderate focal areas of hemorrhage (H) and moderate pyknotic (P) pyramidal cell respectively could be due to the anti-diabetic and healing/ameliorating effects as the leaf extract of *C. papaya* to the diabetic wistar rats. Research has shown that *C. papaya* leaf extract exerts hypoglycemic and antioxidant effect, improved lipid profile in diabetic rats and positively affect integrity and function of both liver and pancreas [54]. Also, the pulp and the other parts of *C. papaya* (leaves and seeds) present antioxidant, anti-hypertensive, hypoglycemic, and hypolipidemic actions, which, in turn, can contribute to the prevention and treatment of obesity and associated metabolic disorders [55]. Also, the leaf extract of *C. papaya* could have improved the histological damages to the cerebellum due to the induced-alloxan. Thus the leaf extract of *C. papaya* ameliorates the cerebellum of the alloxan-induced diabetic wistar rats.

The result of Micrographs 5 and 6 treated with 400mg/kg and 600mg/kg of *N. laevis* leaf extracts showed moderate regeneration with mild vacoulation (V) and mild pyknotic (P) pyramidal cell in GE2.and moderate regeneration moderate increase in the number of

pyramidal cells (PC**)** respectively could be due to anti-diabetic and healing/ameliorating effects as the leaf extract. Research has shown that *N. laevis* flowers and leaves have been used in the treatment of diabetes [39, 40, 42 and 43] respectively, and also has strong antioxidant activity [44]. *N. laevis* leaf possesses the ability of managing hyperglycemia, improve haematological and biochemical derrangements in alloxan induced-diabetic rats, control muscle wasting and induce adipogenesis [45]. Its leaf and stem have anti-diabetic properties [46]. Anaduaka *et al,* [47] reported that the ethanol extracts of the leaves and stem of *N. laevis* possess hepatoprotective properties for curbing oxidative stress complication. Kolawole *et al,* [42] in their research reported that the ethanolic extract of the leaves of *N. laevis* possesses anti-diabetic properties and that it can prevent the complications of diabetes that result from glycation of hemoglobin and lipid peroxidation. The leaf extract of the *N. laevis* has also been reported to lower blood glucose level in diabetic rats [40] and exhibit antioxidant protective properties against rise in oxidative stress and hepatocellular injury in diabetic rat's hepatic tissues at lower dose, indicating that the extract may possess antioxidant activities in diabetics [56].

Micrographs 7 and 8 treated with 200mg/kg of *C. papaya* + 200mg/kg *N. laevis* leaf extract and 300mg/kg of *C. papaya* + 300mg/kg *N. laevis* leaf extract showed mild regeneration with moderate vacoulation (V), pyknotic (P) prymadial cell, mild cytoplasmic ground glass appearance within the molecular layer (ML) and focal area of hemorrhage (FAH) and mild regeneration with moderate vacoulation (V) and moderate fatty changes within the molecular layer (ML) respectively could be due to the combined ant-diabetic and ameliorating effects of both leaf extract. The combined leaf extracts of *C. papaya* and *N. laevis* could have improved the histological and biochemical derrangements to the cerebellum due to the induced alloxan. Research has shown that the combined leaf extracts of *C. papaya* and *N. laevis* have ameliorative effects on the histology of liver of alloxan-induced Wistar rat [57] and exhibit hepatoprotective effects [34, 35]. Thus the combined leaf extracts of *C. papaya* and *N. laevis* ameliorates the cerebellum of the alloxan-induced diabetic wistar rats better than the individual leaf extracts at lower dosages.

5. Conclusion

This study has revealed that combines ethanolic leaf extracts of *C. papaya* and *N. laevis* have anti-diabetic and ameliorating effects on the cerebellum of alloxan-induced male wistar rats. Thus, this therefore supports the use of combined leaf extracts of *C. papaya* and *N. laevis* for the treatment of diabetes mellitus.

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Conflict of interest: None declared.

Ethical Approval: Approved by Institutional ethical approval.

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